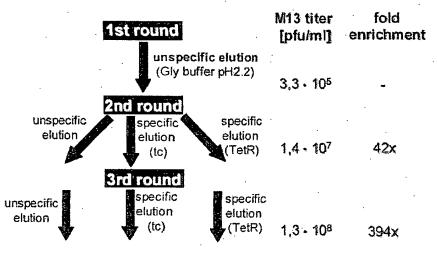
Figure 1: Experimental procedure for the in vitro selection.



Final M13-eluates

Figure 2: Example for in vitro selected sequences.

• Unspecific elution (Gly buffer, pH 2.2)

```
pep1 Trp - His - Gly - Ala - Ile - Leu - Gly - Ser - Ala - Arg - Ala - Gln
pep2 Leu - Pro - Ser - Tyr - Met - Leu - His - Leu - Trp - Ser - Pro - His
pep3 Ala - His - Tyr - Ser - Leu - Tyr - Trp - Pro - Met - Ala - Thr - Phe
pep4 Tyr - His - Asn - Leu - Tyr - Gly - Leu - Pro - Leu - Gly - Pro - Trp
pep5 Trp - His - Gln - Thr - Tyr - Thr - Ser - Ser - Leu - Trp - Glu - Ser
```

Specific elution (TetR, 4μM)

```
pep1 Trp - Thr - Trp - Asn - Ala - Tyr - Ala - Phe - Ala - Ala - Pro - Ser
pep2 Trp - His - Ser - Ser - Phe - Asn - Met - Phe - Ala - Tyr - Pro - Met
pep3 Trp - His - Leu - Pro - Leu - Ser - Trp - Thr - Thr - Arg - Leu - Pro
pep4 Trp - His - Thr - Pro - Ile - Ser - Leu - Leu - Lys - Gln - Val - Arg
pep5 Trp - His - Trp - Thr - Phe - Ser - Ser - Pro - Leu - Met - Gln - Thr
```

Figure 3: Characterisation of TetR-phage binding by ELISA.

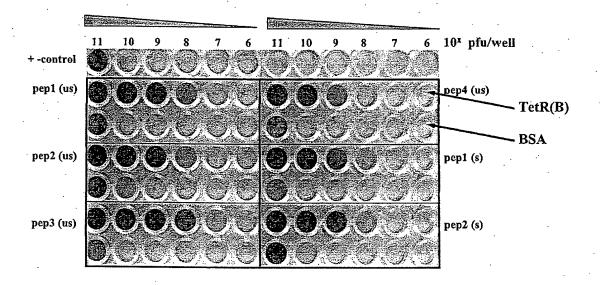
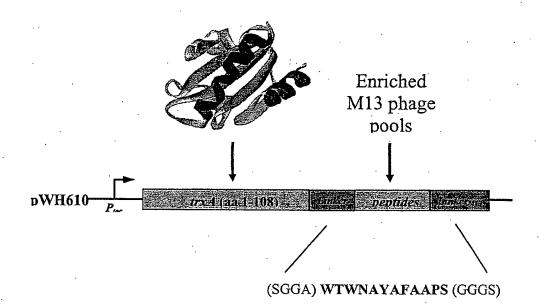


Figure 4: Design of the peptide expressing construct.



5/20 Figure 5: Setup of the *in vivo* screening system.

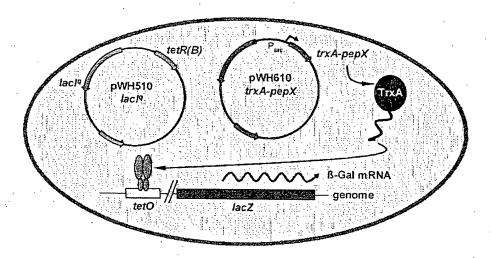
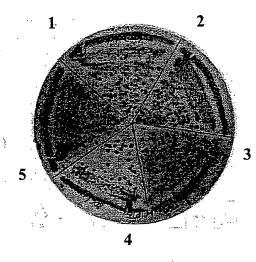
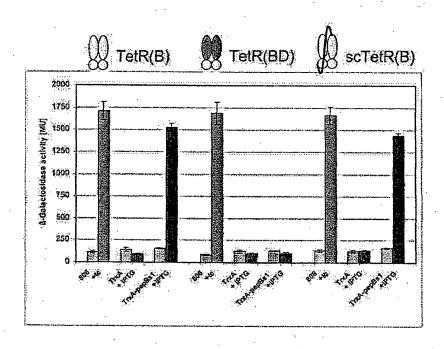


Figure 6: McConkey plate.

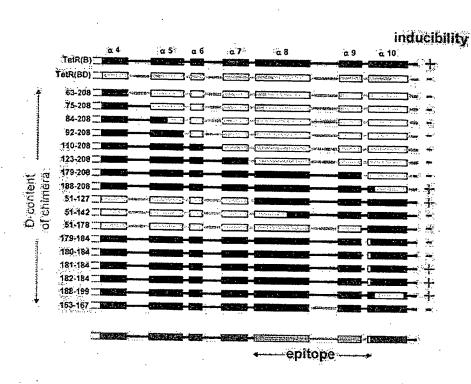


box	Plasmid I* encoding	Plasmid II** encoding	B-Gal activity
1	TetR(B)	TrxA-pepBs1	+
2	TetR(B)	TrxA-pepBs1	+
3	TetR(B)	-	-
4	<u>-</u>	-	+ (100%)
5	TetR(B)	TrxA	-

\* pWH510lacl\* for TetR(B), pWH1200 (Altschmied et al., 1988) pWH610 for TrxA/TrxA-pepBs1, pWH806 (Wissmann et al., 1991) "+" = induced (yellow colonies) "-" = uninduced (colorless colonies)



8/20
Figure 8: Identification of the region of interaction between TetR and TrxA-pepBs1 by in vivo epitope mapping.



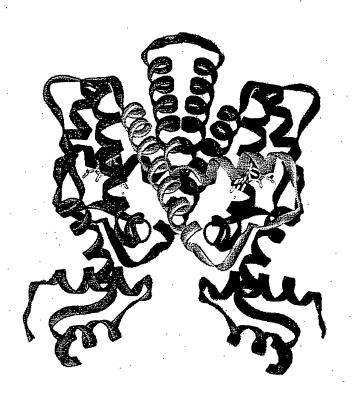
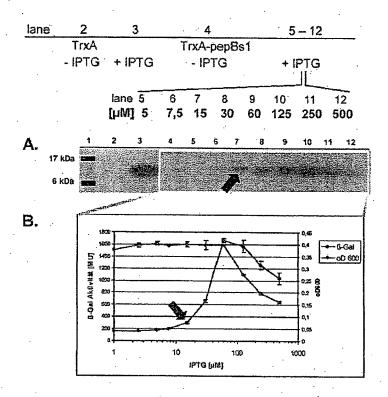
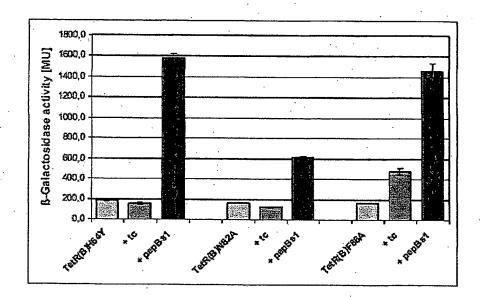


Figure 10: Expression of the peptide correlates with induction of TetR.



11/20 Figure 11: In vivo characterisation of non-inducible TetR mutants.



12/20 Figure 12: Position of the amino acids H64, N82 and F86 relative to tetracycline and the interaction epitope.

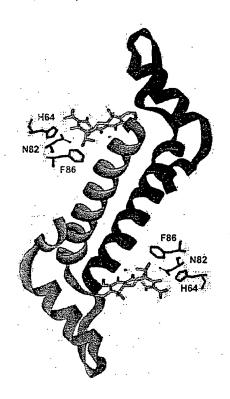


Figure 13: Amino acids contacting tc.

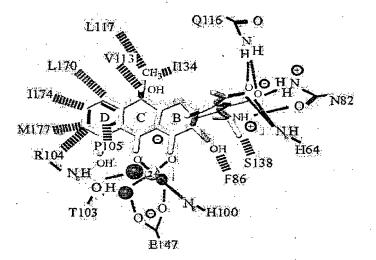


Figure 14: In vivo characterisation of TetR inducibility by TrxA fusion proteins.

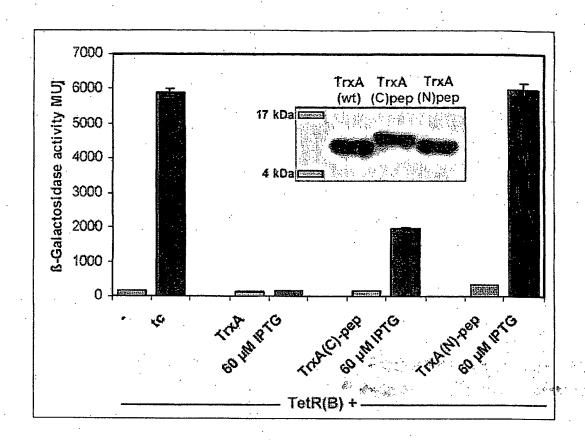


Figure 15: Correlation between the protein level and induction of TetR(B).

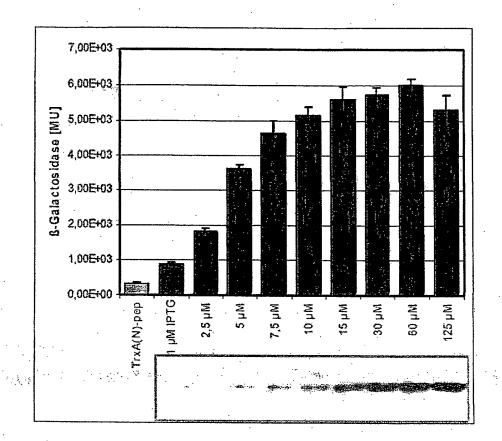
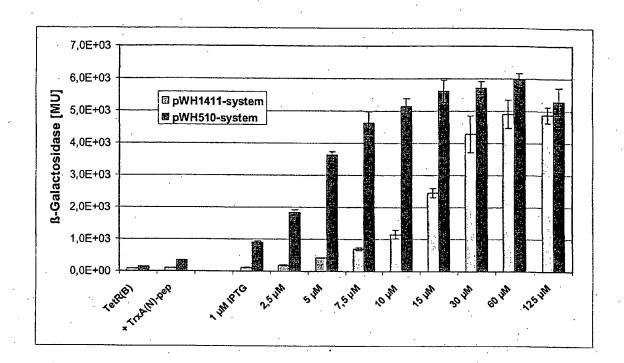


Figure 16: Comparison of a low and high TetR-expressing system.



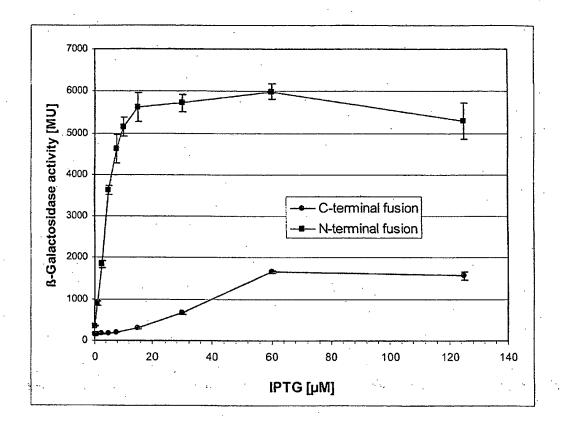


Figure 18: LacZ assay for the TetR-inducing fusion protein SbmC-pepBs1.

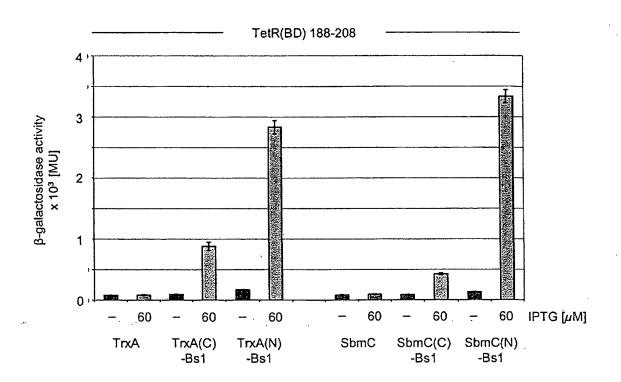


Figure 19: An in-frame fusion of an insertion element (IE<sup>FKS</sup>) encoding the peptide Bs1 to TrxA leads to a protein that induces TetR(B).

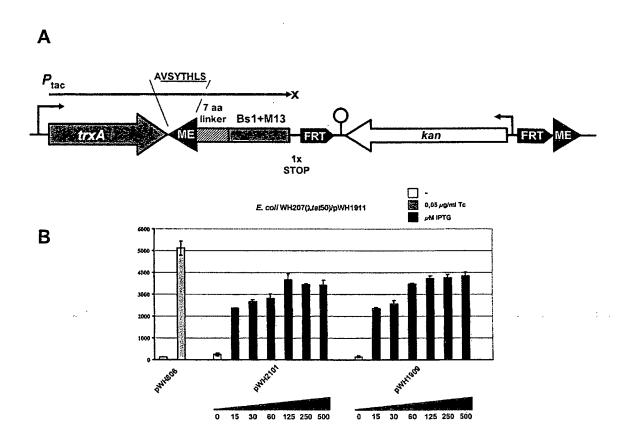


Figure 20: An in-frame fusion of the insertion element IE<sup>FSK</sup> to the *atpD* ORF at its endogenous location in the *E. coli* genome leads to a protein that induces TetR(B).

